

B2 Media osmolality for mammalian cell culture is usually held in the range of 280-300 (Jakoby, W.B., and Pastan, I.H., Methods in Enzymology, vol. LVIII, "Cell Culture", Academic Press (1979), pp. 136-137). Of course, the optimal value may depend upon the specific cell type. For example, as reported in Tissue Culture, Methods and Applications, edited by Kruse, Jr., P.F. and Patterson, Jr., M.K., Academic Press (1973) p. 704, human lymphocytes survive best at low (about 230 milliosmole/kg (mOsmol/kg)0, and granulocytes at higher osmolalities (about 330 mOsmol/kg.) Mouse and rabbit eggs develop optimally in vivo at around 270 mOsmol/kg, 250-280 mOsmol/kg being satisfactory, while above 280 mOsmol/kg development is retarded. Iscove reports 280 mOsmol/kg to be optimum for growth of murine lymphocytes and hemopoietic cells, and Iscove's modified DME is adjusted for this growth promoting osmolality (Iscove, N.N. (1984) Method for Serum-Free Culture of Neuronal and Lymphoid Cells, pp. 169-185, Alan R. Liss, ed., New York.

Please amend the paragraph at page 6, lines 19-24, to read as follows:

B3 As used herein the term "osmolality" refers to the total osmotic activity contributed by ions and non-ionized molecules to a media solution. Osmolality, like molality, relates to weight of solvent (mOsmol/kg H₂O) while osmolarity, like molarity, relates to volume (mOsm/liter solution). Osmolality is one method used to monitor solute stress. Standard osmolality refers to the optimum range of clonal growth of mammalian cells which occurs at 290±30 mOsmol/kg.

Please amend the paragraph beginning at page 9, line 25, continuing through page 10, line 1, to read as follows:

B4 The method of the invention has been shown to increase antibody titer regardless of the presence or absence of serum in the medium. The cell lines used in the present invention may be cell lines of diverse mammalian origin. Rat, mouse, hamster, primate and human embodiments are contemplated, with human and murine embodiments illustrated in the examples which follow. The antibodies may be of any class with IgM and IgG types being specifically

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Conclude

exemplified herein. The human embodiments specifically exemplified herein are the products of triomas synthesized by somatic cell hybridization using a mouse x human parent hybrid cell line and Epstein-Barr virus (EBV)-transformed human peripheral blood lymphocytes (PBLs) or splenocytes from non-immunized volunteers or volunteers immunized with available Gram-negative bacterial vaccines or inactivated Gram-negative bacteria. Fresh PBLs or splenocytes (not transformed) may be used, if desired. A detailed description of the synthesis of the hybridomas, including the fusion protocol, enzyme-linked immunosorbent assays (ELISAs) and hybrid screening procedure, exemplified in the following examples is disclosed in U.S. Serial No. 057,763, supra. The discussion of these procedures is incorporated herein by reference.

Please amend the paragraph at page 10, lines 16-28, to read as follows:

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Following removal of non-adherent cells, a population of cells specifically enriched for the antigen used is obtained. These cells are transformed by EBV and cultured at approximately 10^3 cells per microtiter well using an irradiated lymphoblastoid feeder cell layer. Supernatants from the resulting lymphoblastoid cells are screened by ELISA against an E. coli Rc lipopolysaccharide (LPS) and a Salmonella Re LPS. Cells that are positive for either Rc or Re lipid A LPS are expanded and fused to a 6-thioguanine-resistant mouse x human B cell fusion partner. If the mouse x human B cell fusion partner is used, hybrids are selected in ouabain and azaserine. Supernatants from the Rc or Re positive hybrids are assayed by ELISA against a spectrum of Gram-negative bacteria and purified Gram-negative bacterial LPSs. Cultures exhibiting a wide range of activity are chosen for in vivo LPS neutralizing activity. Many but not all antibodies so produced are of the IgM class and most demonstrate binding to a wide range of purified lipid A's or rough LPS's. The antibodies demonstrate binding to various smooth LPS's and to a range of clinical bacterial isolates by ELISA.

Please amend the paragraph at page 11, lines 16-21, to read as follows: